

244.4 216.9

138.9

104.5

78.2

54.7

33.3

Figure S1: Transfer of pDETAB5. A) Apal-treated genomic DNA from DETAB-E227, ATCC 17978 and putative ATCC 17978 transconjugants after pulsed-field gel electrophoresis. (PFGE) B) Agarose gel showing the products of PCRs targeting the rep genes of pDETAB4 (GR24) and pDETAB5 (GR13). The source of template DNA for each reaction is labelled above, with (-) indicative of a no-DNA control. The sizes in base pairs of labelled DNA size marker bands are indicated to the left. C) S1-treated DNA after PFGE and hybridisation with *bla*_{NDM-1} and *bla*_{OXA-58}-specific probes. The sizes of bands in the DNA size marker (in kilobase pairs) are indicated to the left.

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244.4 216.9

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Figure S2: Circular map of pDETAB13. Plasmid sequence is shown as a black line, with arrows inside representing ORFs. Coloured boxes represent IS. Black lines marked "C/D" or "D/C" represent pdif sites and indicate their orientations. Drawn to scale from GenBank accession CP073061.



Figure S3: GR13 *rep* gene SNP matrix. The numbers of SNPs between *rep* genes and plasmid lineage memberships are indicated by shading as outlined in the legends to the right of the grid.



		Leteet DEI/IB		
Replicon	Plasmid	Primer	Primer sequence (5'-3')	Expected product
type		name		size (bp)
GR24	pDETAB4	Hgz_103-F	TGGCAAGATTGAGGTGGTTC	327
		Hgz_103-R	AAGTTGGTCATATCCGTACTTTCG	
GR13	pDETAB5	GR13-F	TAGTAACCGTCTGATTAGAC	680
		GR13-R	GACCTTTCTTGATGGTATCG	

Table S1: Primers used to detect DETAB-E227 plasmid replicons via PCR

All primers were used in PCRs with annealing temperatures of 55°C.

IS	Closest match (family)	% ID	Plasmid
ISAcsp13	ISAba49 (IS66)	93.7	CP055284
ISAba62	ISAba12 (IS5)	92.6	pDETAB13
ISAba63	IS <i>Alw4</i> (IS <i>3</i>)	94.5	pDETAB13
ISAba64	ISAba26 (IS256)	86.2	pDETAB13
ISAba66	ISAba29 (IS3)	86.7	CP042558
ISAba67	ISAcra1 (IS1595)	88.0	CP042558
ISAba68	ISAha1 (IS5)	92.3	CP041149
ISAba69	ISA/w22 (ISNCY)	91.0	CP033769
ISAba70	ISAjo2 (ISNCY)	92.3	CP038259
ISAjo3	ISAca1 (IS3)	90.8	CP010358
ISAjo4	ISA/w16 (IS66)	94.2	CP079794
ISAjo5	ISAba32 (ISNCY)	92.8	CP068198
ISAjo6	ISAba46 (IS66)	93.8	CP068198
ISApi1	ISAcsp3 (IS3)	94.2	CP027251
ISApi2	ISAjo2 (ISNCY)	89.7	CP033536
ISAps1	ISAba17 (IS66)	88.9	CP055284
ISAso2	ISAjo2 (ISNCY)	93.5	JX101647

89.3 JX101647

ISAlw22 (ISNCY)

ISAso1

Table S2: Novel IS found in the GR13 plasmid collection

Tuble 55.741									
Isolates	-	Antibioti	c ¹ minir	num inh	nibitory	concent	tration (I	ng/L) ²	
	IMP	MEM	CAZ	GEN	тов	LEV	CIP	COL	TGC
DETAB-P39	0.125	0.5	4	0.5	1	0.06	0.125	1	0.25
	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
DETAB-E227	256	128	>256	256	16	4	4	1	0.5
	(R)	(R)	(R)	(R)	(R)	(1)	(R)	(S)	(S)
ATCC17978	0.5	0.25	4	0.5	0.5	0.125	0.125	0.5	0.5
	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)	(S)
ATCC17978	256	256	>256	>256	8	0.125	0.125	0.5	0.25
(pDETAB5) ³	(R)	(R)	(R)	(R)	(1)	(S)	(S)	(S)	(S)

Table S3: Antibiotic minimum inhibitory concentrations

¹ IMP = imipenem, MEM = meropenem, CAZ = ceftazidime, GEN = gentamicin, TOB = tobramycin, LEV = levofloxacin, CIP = ciprofloxacin, COL = colistin, TGC = tigecycline

² minimum inhibitory concentrations classed as resistant (R), intermediate (I) or sensitive (S)

³ transconjugant derived from mating DETAB-E227 with ATCC 17978

	Experiment					
	1	2	3	Mean		
#Donors (D)	1.90×10 ⁹	1.80×10 ⁹	4.2×10 ⁹			
#Transconjugants (TC)	1.75×10 ³	1.65×10 ³	1.05×10 ³			
Conjugation frequency (TC/D)	9.21×10 ⁻⁷	9.17×10 ⁻⁷	2.50×10 ⁻⁷	6.96×10 ⁻⁷		

Table S4: Transfer frequency of pDETAB5 from DETAB-E227 to ATCC 17978

Table S5: GR13 plasmids in GenBank (last search August 9, 2021)

Plasmid	Accession	Host	Country	Year	Source	Size	L	+GR	antibiotic resistance genes
p3ABAYE	CU459140	A. baumannii	France	2001	human clinical	94,413	1	-	-
pMS32-1	KJ616405	A. pittii	China; Taiwan	-	-	94,418	1	-	-
p6411-89.111kb	CP010369	A. nosocomialis	Colombia	2012	-	89,111	1	-	-
unnamed2	CP014653	Acinetobacter sp.	China; Panjin	2015	marine sediment	50,047	1	-	-
p2012N21-164-1	CP033536	A. pittii	China; Taiwan	2012	human	97,329	1	-	-
pC54_002	CP042366	A. pittii	Australia	2014	human clinical	76,008	1	-	-
p1_100020	CP027251	A. pittii	China; Chengdu	2015	human	77,340	1	-	-
p1_100004	CP027247	A. pittii	China; Chengdu	2015	human	66,765	1	-	-
pAP43-2	CP043054	A. pittii	China; Hangzhou	2018	human urine	92,276	1	-	-
unnamed3	CP069540	A. pittii	Germany	≤1994	human urine	94,387	1	-	-
unnamed2	CP069506	A. pittii	unknown	≤1994	human sputum	94,379	1	-	-
pAS61-1	CP061590	A. seifertii	China; Taiwan	2010	human blood	93,205	1	-	-
p2014N21-145-2	CP033570	A. pittii	China; Taiwan	2014	human	72,034	1	-	-
p2014S07-126-2	CP033532	A. pittii	China; Taiwan	2014	human	96,775	1	-	-
unnamed1	CP077304	A. pittii	Germany	≤1994	human ear discharge	94,386	1	-	-
p1_005069	CP026087	A. pittii	China; Chengdu	-	human	91,563	1	-	-
pDETAB5	CP072528	A. baumannii	China; Hangzhou	2019	intensive care unit environment	97,035	2	-	<i>bla</i> _{NDM-1} , <i>ble</i> _{MBL} , <i>bla</i> _{OXA-58} , <i>aacC2d</i> , <i>msr</i> (E)- <i>mph</i> (E), <i>sul2</i>
pM131-2	JX101647	A. soli	China; Taiwan	2010	human sputum	84,995	2	-	bla _{OXA-58} , aphA1, aacC2d, sul2 (x2)
p255n_1	KT852971	A. baumannii	Vietnam	2005	human nasal	92,939	2	-	blaoxA-58, aacC2d, blaveB, arr-2, aadA1, aadB, cmlA6, msr(E)-mph(E),
									sul1, sul2
pNDM1_010045	CP028560	Acinetobacter sp.	China; Chengdu	2015	sewage	190,170	2	34	$bla_{\text{NDM-1}}, ble_{\text{MBL}}, bla_{\text{OXA-58}}(x2), aacC2d, floR, msr(E)-mph(E), sul2,$
									merA-2
pOXA58_005078	CP027245	A. baumannii	China; Chengdu	-	human	70,509	2	-	<i>bla</i> _{OXA-58} , <i>aacC2d</i> , <i>floR</i> , <i>msr</i> (E)- <i>mph</i> (E)
pBspH2	CP055284	Acinetobacter sp.	USA	1986	soil	161,809	3	-	-
unnamed3	CP068198	A. johnsonii	The Netherlands	2008	spacecraft-associated clean room	206,659	3	-	•
plas1	CP070867	A. johnsonii	China; Shanghai	2018	bigeye tuna	149,408	3	-	-
pAJ_082-3	CP079794	A. johnsonii	Pakistan	2016	intensive care unit sink	155,957	3	-	-
unnamed1	CP033769	A. baumannii	USA	2016	human sputum	97,783	-	-	-
pTS134338	CP042210	A. baumannii	India	2005	soil	134,338	-	-	-
pOCUAc17-1	AP024799	A. pittii	Japan*	≤2021	human blood*	69,156	-	-	-
unnamed1	CP077233	A. pittii	Germany	≤1990	human wound swab	69,574	-	-	-
pEC_gr13	CP038263	A. baumannii	Czech Republic	2018	frozen turkey liver	128,013	-	-	-
pE47_002	CP042558	A. baumannii	Australia	2013	hospital environment	59,744	-	-	-
pDETAB13	CP073061	A. baumannii	China; Hangzhou	2019	human rectal	91,083	-	-	-
pXBB1-8	CP010358	A. johnsonii	China; Chengdu*	-	sewage*	117,483	-	-	-
pCUVET596	CP041149	A. baumannii	Thailand	2017	dog urine	82,016	-	-	-
p2012C01-137-2	CP033559	A. nosocomialis	China; Taiwan	2012	human	72,978	-	-	-
unnamed1	CP068175	A. ursingii	The Netherlands	2003	spacecraft-associated clean room	120,510	-	-	-
pAR3	CP038025	A. radioresistans	Chile	2008	soil	80,103	-	-	-
p2014S06-099-1	CP033541	A. pittii	China; Taiwan	2014	human	125,715	-	-	-
pIC001A	CP022299	A. johnsonii	Japan	2003	Tokyo Bay water	94,476	-	26	-
pCAM180A	CP044357	A. baumannii	Cambodia	2016	human oral	92,034	-	-	-
p2010S01-197-2	CP033563	A. nosocomialis	China; Taiwan	2010	human	92,044	-	-	$bla_{OXA-58}(x2), aacC2d, sul2$
unnamed2	CP069498	A. pittii	Germany	≤1994	human blood	128,321	-	24	-
pEH_Gr13	CP038259	A. baumannii	Czech Republic	2018	human tracheal	135,229	-	_	

* = uncertain, information derived from submitter information and expected publication titles included in GenBank entries

L = lineage defined here, plasmids assigned to lineages 1, 2 or 3 are shaded pink, blue or orange, respectively

+GR = plasmids contain an additional replicon of GR type #

modulo	cizo	nutative function or content	roforonco
mouule	SIZE (ha)	putative function of content	reference
	(qa)		plasmid
oxa58	2,257	carbapenem resistance	pDETAB5
sul2	5,051	sulphonamide resistance	pDETAB5
msrE-mphE	2,950	macrolide resistance	pDETAB5
aacC2d	9,468	aminoglycoside resistance	pDETAB5
<i>dif</i> -696a	696	toxin-antitoxin	pDETAB5
<i>dif</i> -696b	696	toxin-antitoxin	pDETAB13
dif-823	823	toxin-antitoxin (AbkAB)	pDETAB5
dif-701	701	toxin-antitoxin (HigAB)	pDETAB5
<i>dif</i> -1491	1,491	toxin-antitoxin + unknown ORFs	pDETAB13
dif-2111	2,111	alcohol tolerance	pDETAB13
dif-6874	6,874	alcohol tolerance	AP024799
dif-7136	7,136	alcohol tolerance	CU459140
dif-28327	28,327	copper resistance	CP068175
<i>dif</i> -RND	9,903	RND efflux	pDETAB5
dif-7932	7,932	metabolism; possibly aromatic compound degradation	CP022299
dif-1769	1,769	sulphate permease	CP022299
<i>dif-</i> 6620	6,620	small plasmid + mobilisation determinant	CP022299
<i>dif</i> -801	801	VRR-NUC domain protein	CP022299
dif-614	614	unknown	pDETAB5
dif-1209	1,209	unknown	pDETAB5
<i>dif</i> -1612	1,612	unknown	pDETAB13

Table S6: Characteristics of *dif* modules examined in this study